Evidence of major genes affecting stress response in rainbow trout using Bayesian methods of complex segregation analysis

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Evidence of major genes affecting stress response in rainbow trout using Bayesian methods of complex segregation analysis[1]

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ABSTRACT: As a first step toward the genetic mapping of QTL affecting stress response variation in rainbow trout, we performed complex segregation analyses (CSA) fitting mixed inheritance models of plasma cortisol by using Bayesian methods in large full-sib families of rainbow trout. To date, no studies have been conducted to determine the mode of inheritance of stress response as measured by plasma cortisol response when using a crowding stress paradigm and CSA in rainbow trout. The main objective of this study was to determine the mode of inheritance of plasma cortisol after a crowding stress. The results from fitting mixed inheritance models with Bayesian CSA suggest that 1 or more major genes with dominant cortisol-decreasing alleles and small additive genetic effects of a large number of independent genes likely underlie the genetic variation of plasma cortisol in the rainbow trout families evaluated. Plasma cortisol is genetically determined, with heritabilities of 0.22 to 0.39. Furthermore, a major gene with an additive effect of −42 ng/mL (approximately 1.0 genetic SD) is segregating in this rainbow trout broodstock population. These findings provide a basis for designing and executing genome-wide linkage studies to identify QTL for stress response in rainbow trout broodstock and markers for selective breeding.

Key words: Bayesian analysis, major gene, plasma cortisol, rainbow trout, segregation analysis, stress response

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INTRODUCTION

Improved resistance to stress has been identified as a target for genetic improvement in rainbow trout (*Oncorhynchus mykiss*) because stress has been reported to have negative effects on important production traits, including growth, feed efficiency, reproductive performance, and disease resistance (Pottinger and Pickering, 1997; Wendelaar Bonga, 1997; Barton, 2002; Portz et al., 2006). Disappointingly, studies to date have identified only weak associations between stress response measures and performance traits (e.g., Fevolden et al., 1992; Trenzado et al., 2003; Weber et al., 2008). This failure of altered stress responses, including cortisol responsiveness, to have a consistent effect on performance phenotypes known to be affected by stress may be due in part to the many possible physiological and genetic bases for variation in each measure of stress responsiveness, including cortisol responsiveness.

The main objective of this study was to determine the mode of inheritance of plasma cortisol by using Bayesian methods of complex segregation analysis (**CSA**) in large full-sib (**FS**) families. The CSA is a statistical method to determine whether a mixed mode of inheritance of a major gene (**MG**) and polygenic background effects is consistent with the inheritance of a trait when using only phenotypic data (no marker data are included; Guo and Thompson, 1992; Thaller et al., 1996; Janss et al., 1997). We evaluated whether a mixed model of inheritance including an MG and polygenic background effects was consistent with the inheritance of plasma cortisol. This study provides the basis for designing and carrying out genome-wide linkage scans for stress response QTL in rainbow trout. In addition, markers for genotype variation at the QTL are sought to resolve crude phenotypic measures of stress responsiveness, such as plasma cortisol, into more discrete genotypes, which should associate more strongly

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with performance traits (e.g., disease resistance) than do the phenotypic measures.

MATERIALS AND METHODS

All animal handling procedures were reviewed and approved by the National Center for Cool and Cold Water Aquaculture (**NCCCWA**) animal care and use committee.

Animals and Measured Traits

The founder NCCCWA population included rainbow trout from 3 different strains (Silverstein et al., 2004). The first parental generation (P_0, 2002 spawn) included 100 families that were evaluated for growth performance. From these P_0 families, we evaluated the stress response in 64 families. We selected families with high growth performance and low cortisol response (Weber and Silverstein, 2007). High cortisol response is positively correlated with growth; when we looked at the fish that were to be bred based on growth performance, there were few fish with low cortisol. Therefore, we kept some of the low-cortisol fish and bred them so that we had a range in cortisol response beyond that of the selected high-growth fish.

In the second parental generation (P_1, 2004 spawn), we generated 111 families, which were evaluated for growth performance and resistance to *Yersinia ruckeri*. From these P_1 families, 60 families were evaluated for stress response when the fish were approximately 130 g of BW by using 4× repeated measurements per fish (Weber et al., 2008). In this P_1 generation, we selected families for high growth performance and low cortisol response. From these evaluations on stress response, parents with high and low plasma cortisol response were selected as breeders to produce the FS families evaluated in this study (high × low and low × high families).

Twelve large FS families (F_1, 2006 spawn), which included 3 paternal half-sib groups, of rainbow trout (*O. mykiss*) produced at the NCCCWA were used in this study. The most informative designs for segregation analysis have a collection of medium-sized connected FS families from an outbred population so that the different mating types will be present. By connections between families, the Mendelian segregation of a putative MG can be further confirmed. From each of the FS families, a random sample of 40 siblings per FS family was assessed for plasma cortisol response after a crowding stress. Each individual was measured at 4 time points during this study. The first time evaluation was performed when the fish were approximately 160 g of BW. The subsequent time point evaluations were spaced at approximately 4-wk intervals.

Plasma cortisol response after a crowding stress was measured in each FS family offspring fish at 4 time points by using the protocol outlined below. We recorded BW and length for each of the offspring fish at each sampling period. During the fourth repeated measurement, we recorded the fish that were ripe (<5%), which were those that released milt or eggs when pressure was applied to the abdomen.

Crowding Stress Challenge and Measurement of Plasma Cortisol

The procedures for the crowding stress challenge followed those described by Weber and Silverstein (2007), as modified from Pottinger and Carrick (1999b). The crowding stress challenge for the parents was described in Weber et al. (2008), and the challenge for the offspring is described here. A total of 480 offspring (40 from each of 12 FS families) were included at the beginning of the study; all hatched within a 14-d span. Fish were reared as individual families. Fish were exposed to an artificial ambient photoperiod and reared in continuous-flow groundwater with an ambient temperature of approximately 11.5 to 13.5°C and a dissolved oxygen content near saturation. The fish from each of the families were placed in 120-L blue polypropylene tanks at approximately 1 mo of age. Seven weeks before the beginning of the experiment, fish were tagged with passive integrated transponder tags (Avid Identification Systems Inc., Norco, CA) and the fish from each family were split into 5 tanks of 8 fish each. The locations of the tanks were determined using a random number-generating program (C++ script written by R. L. Vallejo, unpublished program). Fish in the stress study were fed Zeigler Gold (Zeigler Bros. Inc., Gardners, PA) at 2% of BW/d. The 8 fish from each tank were sampled 4 times at 4-wk intervals beginning September 5, 2004, when the fish were approximately 160 g of BW. Fish were not fed the day of sampling or the afternoon preceding the sampling. Sampling began at 0930 h and finished at 1540 h for 2 d in each sampling period. The fish were returned to different tanks after sampling, and the order for sampling families was changed each sampling period.

For the crowding stress challenge, at 10-min intervals fish from a single family were netted and transferred from a 120-L tank to a 6- or 15-L tank and were left undisturbed for 3 h. Fish were transferred to 6-L tanks in the first sampling period and to 15-L tanks in the remaining sampling periods. All tanks had flow-through water. After 3 h in the crowding tank, the fish were netted and transferred into an anesthesia bath with 100 mg/L of tricaine methanesulfonate (tricaine-S; Western Chemical Inc., Ferndale, WA). Within 5 min of the fish entering the anesthesia, a 0.5-mL sample of blood was collected from each fish by caudal puncture by using heparinized syringes (heparin 10 mg/mL) with 23-gauge needles. Blood samples were centrifuged at 10,000 × *g* for 10 min at 4°C to separate plasma, and the plasma was stored at −80°C until analyzed for cortisol concentration. Body weight was measured to the nearest 1 g, and fork length was measured to the nearest 0.1 cm, after bleeding. To perform plasma cortisol measurements,

Table 1. Variables with a significant contribution to the predictive power of plasma cortisol models[1] after stepwise selection with the REG procedure[2]

		F-value ($P > F$)		
Variable	Type of effect	Model A	Model B	Model C
Family	Random	25.2 (<0.0001)	NI[3]	NI
Animal	Random	5.0 (0.0254)	NI	NI
Sire	Random	128.4 (<0.0001)	NI	NI
Dam	Random	NS[4]	NI	NI
Tank	Fixed	NS	NS	NI
Time	Fixed	88.4 (<0.0001)	95.2 (<0.0001)	NI
BW	Continuous covariate	NS	7.2 (0.0075)	40.77 (<0.0001)
Length	Continuous covariate	97.1 (<0.0001)	11.9 (0.0006)	NS
Ripe	Fixed	NS	NS	4.04 (0.0446)

[1]Variables included in fitted models: model A: family, animal, sire, dam, tank, time, BW, length, and ripe; model B: tank, time, BW, length, and ripe; model C: BW, length, and ripe.

[2]The stepwise selection model was performed with the REG procedure (SAS Inst. Inc., Cary, NC) by using significance level (SL) of SLENTRY = 0.50 and SLSTAY = 0.05.

[3]NI indicates this variable was not included in the model to fit.

[4]NS indicates this variable had a nonsignificant ($P > 0.05$) contribution to plasma cortisol.

the plasma samples were heated to 100°C for 15 min in glutamate buffer (pH 3.3) to denature binding proteins. Plasma cortisol was measured (ng/mL) by tritium RIA according to procedures described by Redding et al. (1984). The assays for the cortisol measurements of the parents used a cortisol antiserum purchased from Esoterix Inc. (cortisol F3-314, lot 345; Esoterix Inc., Calabasas Hills, CA) as validated in the studies mentioned. The assays for the cortisol measurements of the offspring were conducted using cortisol antiserum (R4866; provided by Coralie Monroe, University of California-Davis, School of Veterinary Medicine, Department of Reproduction), which was validated for use in rainbow trout (Barry et al., 1993).

Statistical Analysis of Nongenetic Effects

The analysis included 12 FS families (n = 503 individuals, 23 parents and 480 offspring) with 4 repeated measurements of plasma cortisol per individual. The mean plasma cortisol was 58.08 ± 33.25 ng/mL, with a total of 1,895 data records. As expected, we found that the 4 repeated measurements of plasma cortisol were highly correlated [n = 503 individuals (parents and offspring), Pearson's r = 0.22 to 0.55, $P < 0.0001$] using the CORR procedure (SAS Inst. Inc., Cary, NC). Subsequently, we decided to fit a permanent environmental effect in the polygenic and mixed inheritance models used in CSA of plasma cortisol to account for the covariance between the records of an individual (i.e., repeated measurements).

Before performing CSA of plasma cortisol, to identify significant predictors of plasma cortisol response, we performed multivariable regression analysis by using 3 linear models (Table 1) that included random (family, animal, sire, and dam) and fixed (tank, time, and ripe) effects and continuous covariates (BW and length) by STEPWISE model selection with the REG procedure of SAS. Model A included all 9 variables; model B included fixed effects and continuous covariates (tank, time, ripe, BW, and length); and model C included fish measurements such as length, BW, and ripe. Overall, the STEPWISE model selection indicated that the effects of time ($P < 0.0001$), BW ($P \leq 0.0075$), length ($P \leq 0.0006$), and ripe ($P = 0.045$) on plasma cortisol were significant (Table 1). The fixed effect of tank was a nonsignificant ($P > 0.05$) predictor of plasma cortisol. Subsequently, we decided to include the variables time, BW, length, and ripe as covariates in the mixed inheritance models used in CSA of plasma cortisol to minimize the variance in the sampled population.

Polygenic Model

The pure polygenic model was used to supply an overall quantification of genetic variance for the trait analyzed. The model postulating pure polygenic inheritance was specified as

$$\mathbf{y_{n\times 1}} = \mathbf{X_{n\times q}b_{q\times 1}} + \mathbf{Z_{n\times r}u_{r\times 1}} + \mathbf{Z_{n\times r}p_{r\times 1}} + \mathbf{e_{n\times 1}}, \quad [1]$$

where $\mathbf{y_{n\times 1}}$ is the vector of observations, where $\mathbf{n}$ is the number of records; $\mathbf{b_{q\times 1}}$ is the vector of nongenetic effects (time, BW, length, and ripe), where $\mathbf{q}$ is the number of levels for nongenetic effects; $\mathbf{u_{r\times 1}}$ is the vector of polygenic effects, where $\mathbf{r}$ is the number of levels for random animal effects; $\mathbf{p_{r\times 1}}$ is the vector of permanent environmental effects; $\mathbf{e_{n\times 1}}$ is the vector of random residual effects; and $\mathbf{X_{n\times q}}$ and $\mathbf{Z_{n\times r}}$ are incidence matrices connecting the unknowns in $\mathbf{b}$, $\mathbf{u}$, and $\mathbf{p}$ to the observations. The parameters of interest for statistical inference in the polygenic model were polygenic variance $\left(\sigma_u^2\right)$, permanent environmental variance $\left(\sigma_p^2\right)$, error variance $\left(\sigma_e^2\right)$, and polygenic heritability, defined as $h_p^2 = \sigma_u^2 \Big/ \left(\sigma_u^2 + \sigma_e^2\right)$.

CSA

Janss et al. (1995) have presented a Bayesian approach for CSA in livestock species. In this study, we used a Bayesian method for CSA implemented in the statistical package iBay, version 1.44 (Janss, 2008).

Mixed Inheritance Model. For analyses of the presence of MG affecting plasma cortisol, a mixed inheritance model was used with nongenetic effects, the effect of 1 MG, effects of background polygenes, and permanent environmental effects. The mixed inheritance model applied to plasma cortisol was

$$\mathbf{y_{n\times 1}} = \mathbf{X_{n\times q}b_{q\times 1}} + \mathbf{Z_{n\times r}u_{r\times 1}} + \mathbf{Z_{n\times r}p_{r\times 1}} + \mathbf{Z_{n\times r}W_{r\times 3}g_{3\times 1}} + \mathbf{e_{n\times 1}}, \quad [2]$$

with all specifications equal to those defined for model 1, and where $\mathbf{W_{r\times 3}}$ is the incidence matrix for MG genotypes, $\mathbf{g_{3\times 1}}$ is the vector of genotype means, and $\mathbf{X_{n\times q}}$ and $\mathbf{Z_{n\times r}}$ are incidence matrices connecting the unknowns in **b**, **u**, **p**, and **Wg** to the observations.

The prior distributions for **u**, **p**, and **e** were assumed as follows: polygenic effects were assumed distributed as $\mathbf{u} \sim N\left(0, \mathbf{A}\sigma_u^2\right)$, where **A** is the numerator relationship matrix; permanent environmental effects were assumed distributed as $\mathbf{p} \sim N\left(0, \mathbf{I}\sigma_p^2\right)$, where **I** is the identity matrix; and errors were assumed distributed as $\mathbf{e} \sim N\left(0, \mathbf{I}\sigma_e^2\right)$. Specification of the statistical model for the Bayesian approach was completed by specifying the use of uniform prior distributions on $\left[-\infty, \infty\right]$ for nongenetic effects and effects at the major locus, uniform prior distributions on $\left[0, \infty\right]$ for variance components, and uniform prior distributions on [0, 1] for allele frequencies.

The MG effect is assumed to result from segregation at a single locus with 2 alleles (A_1,A_2). The alleles A_1 and A_2 are associated with decreased and increased plasma cortisol concentrations, respectively. The frequency of A_1 and A_2 in the sample population was assumed to be p and $q = (1 - p)$, respectively, under the assumption of the Hardy-Weinberg equilibrium. With 3 genotypes distinguished (A_1A_1,A_1A_2,A_2A_2), genotype means were $\mathbf{g}' = (-a, d, a)$, where a and d refer to as the additive and dominant effect, respectively, at the major locus.

The mixed inheritance model included 3 transmission probabilities $\left(\tau_{A_1/A_1A_1}, \tau_{A_1/A_1A_2}, \tau_{A_1/A_2A_2}\right)$, which are defined as the probability that a parent with any of the 3 genotypes (A_1A_1,A_1A_2,A_2A_2) would transmit the allele A_1 to its offspring. In the analysis, the mixed inheritance model was analyzed under Mendelian and non-Mendelian assumptions. If Mendelian inheritance was assumed, the transmission probabilities were fixed to $\tau_{A_1/A_1A_1} = 1$, $\tau_{A_1/A_1A_2} = 0.5$, and $\tau_{A_1/A_2A_2} = 0$. Under non-Mendelian assumptions (the general and general fixed models described below), the transmission probabilities were given a flat prior for parameter estimation.

Gibbs Sampling. Statistical inference was based on a Bayesian approach computing marginal posterior densities of the unknown parameters by the Markov chain Monte Carlo (**MCMC**) method, known as Gibbs sampling (Gelman et al., 2004). The theory and methodology of Bayesian segregation analysis are explained in more detail by Janss et al. (1995), Sorensen (1996), and Janss (2008). The Bayesian approach for CSA allowed the estimation of marginal posterior distributions for nongenetic effects, genotypic values from $\mathbf{g}' = (-a, d, a)$, low phenotype allele A_1 frequency (p), polygenic variance $\left(\sigma_u^2\right)$, residual variance $\left(\sigma_e^2\right)$, and permanent environmental variance $\left(\sigma_p^2\right)$ for plasma cortisol. On the basis of the MG allele effects (a and d) and the allele frequencies (p and q), the additive $\left(\sigma_a^2\right)$, dominant $\left(\sigma_d^2\right)$, and total MG variance $\left(\sigma_g^2\right)$ were calculated as $\sigma_g^2 = \left(\sigma_a^2 + \sigma_d^2\right) = 2pq\left[a + d(q - p)\right]^2 + \left(2pqd\right)^2$ (Falconer and Mackay, 1996). In addition, the proportion of total variance attributable to polygenic effects $\left[R_u = \sigma_u^2 \Big/ \left(\sigma_g^2 + \sigma_u^2 + \sigma_p^2 + \sigma_e^2\right)\right]$, the proportion of total variance attributable to the total effect of an MG $\left[R_g = \sigma_g^2 \Big/ \left(\sigma_g^2 + \sigma_u^2 + \sigma_p^2 + \sigma_e^2\right)\right]$, the proportion of total variance attributable to the additive effect of an MG $\left[R_a = \sigma_a^2 \Big/ \left(\sigma_g^2 + \sigma_u^2 + \sigma_p^2 + \sigma_e^2\right)\right]$, the proportion of total variance attributable to genetic effects $\left[R_{gu} = \left(\sigma_g^2 + \sigma_u^2\right) \Big/ \left(\sigma_g^2 + \sigma_u^2 + \sigma_p^2 + \sigma_e^2\right)\right]$, and the additive effect of an MG expressed in genetic standard deviation units $\left[a_{GSD} = a \Big/ \left(\sigma_g^2 + \sigma_u^2\right)^{1/2}\right]$ were estimated and their marginal posterior distributions were computed.

After exploratory analyses of our data set, the length of each chain was set to 1,200,000 iterations. The first one-half of each chain was discarded (burn-in period of 600,000 iterations) to diminish the effect of the beginning distribution and to allow convergence of the Gibbs sampler (Gelman et al., 2004). On the remaining one-half of the chain (600,000 iterations), a sample was saved from every 10,000 iterations (thinning parameter k = 10,000) to ensure that virtually independent samples were collected. Estimation of posterior distributions of parameters for each model was based on at least 30 replicated Gibbs chains. This Gibbs sampler

allowed approximately 1,800 independent samples to be collected per evaluated model.

Postanalysis and Statistical Inference. Convergence of the Gibbs sampler was judged by using the approximately 1,800 independent samples generated from 30 chains in ANOVA testing for a significant chain effect. Significant differences between chains indicated that the Gibbs sampler had not converged and that generated samples were not from the correct posterior distribution. Convergence was also tested using the criterion outlined by Gelman et al. (2004). For each unknown parameter in the model, a scale reduction factor $\left(\hat{R}\right)$, which involves variance between and within chains, was computed. The $\hat{R}$ can be interpreted as the factor by which the scale of the marginal posterior distribution of each variable would be reduced if the simulations were continued in the limit $n \to \infty$. The $\hat{R}$ should be approximately 1.0 (or at most ≤ 1.1) to indicate convergence of the iterative simulation for the unknown parameter.

Statistical inferences were based on summarizing the generated samples in the form of estimated marginal posterior distributions. As features of the marginal posterior distributions, estimated means and SD were computed. Posterior means were used as point estimates for the parameters. Statistical inferences focused on the genetic variance components $\left(\sigma_u^2, \sigma_g^2\right)$ to determine significance of the MG in the model. Judgments were also based on the shapes of estimated posterior distributions of variance components (Janss et al., 1995), where a nonsignificant variance shows a distribution with global mode at $\sigma^2 = 0$ and a significant variance shows a global mode at $\sigma^2 > 0$. Major gene variance was concluded to be significant when the global mode had a density 20 times larger than the density at $\sigma_g^2 = 0$. Once significant MG variance was found, further inferences focused on the effects at the major locus and on estimable functions of allele frequencies. The highest posterior density region at 95% ($\mathbf{HPDR_{95}}$) was also calculated from the marginal posterior distributions for each unknown parameter.

Mode of Inheritance for Plasma Cortisol. We determined the likely mode of inheritance for plasma cortisol by defining 8 mixed inheritance models and submodels or nested models. The models were categorized into 4 model sets: 1) non-Mendelian (unrestricted τ), which assumes non-Mendelian segregation and which included the general model that maximizes σ_u^2 (model 1) and the general model with fixed $\sigma_u^2 = 0$ (model 2); 2) sporadic, which assumes no genetic effects or the absence of MG and polygenic effects $\left(p = 0, \sigma_u^2 = 0\right)$ and which is equivalent to the assumption of equal transmission probabilities $\left(\tau_{A_1/A_1A_1} = \tau_{A_1/A_1A_2} = \tau_{A_1/A_2A_2}\right)$ (model 3); 3) polygenic, which assumes no MG but a polygenic background (model 4); and 4) Mendelian mixed inheritance of an MG and the polygenic background, which is divided into 4 models: dominance of the cortisol-increasing allele A_2; additive; dominance of the cortisol-decreasing allele A_1; and codominant (models 5 to 8).

When comparing nested models, the larger model has the advantage of making more sense and fitting the data better but has the disadvantage of being more difficult to understand and compute (Gelman et al., 2004). In the model comparisons, we combined the use of Bayes factors (**BF**) with approaches that measure the distance of the data to each of the approximate models by comparing nested models. Let θ be the vector of parameters in the smaller model and ψ be the additional parameters in the expanded model. We then compared the 2 posterior distributions, $p(\theta \mid y)$ and $p(\theta,\psi \mid y)$, along with their predictive distributions for replicated data chains.

The $\log_e$ of the marginal distribution of the data under each model, $\log_e [p(y/H_i)]$, is estimated, which is basically the normalization constant for the marginalized posterior density (Janss, 2008). Here, given 2 competing models, H_1 and H_2, we estimated BF $(H_2; H_1) = p(y/H_2)/p(y/H_1)$, which is the ratio of the marginal likelihood under 1 model to the marginal likelihood under a second model (Gelman et al., 2004). In this study, the guidelines provided by Janss (2008) were used to assess the amount of evidence in the data that supported the difference between compared models, BF $(H_2; H_1)$.

Inference on Transmission Probabilities. After determining the most likely mode of inheritance for plasma cortisol, we analyzed an MG model under non-Mendelian assumptions, defined as

$$\mathbf{y_{n\times 1}} = \mathbf{X_{n\times q}b_{q\times 1}} + \mathbf{Z_{n\times r}p_{r\times 1}} + \mathbf{Z_{n\times r}W_{r\times 3}g_{3\times 1}} + \mathbf{e_{n\times 1}}, \quad [3]$$

with the specifications equal to those defined for model 2. The parameters a, d, and p were fixed to the values estimated for the best-fitting mixed inheritance model, the polygenic variance was set to $\sigma_u^2 = 0$, and the transmission probabilities $\left(\tau_{A_1A_1}, \tau_{A_1A_2}, \tau_{A_2A_2}\right)$ were given a flat prior for parameter estimation (best-fitting MG model). After running the Gibbs sampler as specified previously, the parameters $\mu, \sigma_g^2, \sigma_e^2, \sigma_{pe}^2$ and transmission probabilities $\tau_{A_1A_1}, \tau_{A_1A_2}$, and $\tau_{A_2A_2}$ were estimated from the marginal posterior distributions of the unknown parameters (MG fixed model). For comparison purposes, we also analyzed an MG general model (general fixed model) with all the above parameters treated as unknowns $\left(a, d, p, \tau_{A_1/A_1A_1}, \tau_{A_1/A_1A_2}, \tau_{A_1/A_2A_2}, \mu, \sigma_g^2, \sigma_e^2, \sigma_{pe}^2\right)$ and with

Table 2. Estimated marginal posterior mean, SD, and 95% highest posterior density region for variance components[1] of plasma cortisol using polygenic inheritance model in Bayesian segregation analysis[2]

Statistic	σ_e^2	σ_u^2	σ_p^2	h_p^2
Mean	617.61	223.89	131.66	0.26
SD	23.11	50.88	31.36	0.04
Left $HPDR_{95}$[3]	573.29	128.96	70.55	0.18
Right $HPDR_{95}$	664.58	325.53	193.48	0.35
Within-chain σ^2	537.77	2,586.06	984.57	0.00
Between-chain σ^2	307.46	2,774.66	926.53	0.00
Effective number of samples	3,148	1,678	1,913	1,734
$\hat{R}$[4]	1.00	1.00	1.00	1.00

[1]Variance components: error variance, σ_e^2; polygenic variance, σ_u^2; permanent environmental variance, σ_p^2. h_p^2 is polygenic model heritability, defined as $\sigma_u^2 / \left(\sigma_u^2 + \sigma_e^2\right)$.

[2]Bayesian segregation analysis of plasma cortisol (ng/mL) was performed with the computer application iBay, version 1.44 (Janss, 2008). The Gibbs sampler had these characteristics: number of iterations per chain = 1,200,000; burn-in period = 600,000; thinning = 10,000; collected samples per chain = 60; total chains >30; and total collected samples >1,800.

[3]$HPDR_{95}$ is the 95% highest posterior density region.

[4] $\hat{R}$ is the scale reduction factor. The $\hat{R}$ should be approximately 1.0 (or at most ≤1.1) to indicate convergence of the iterative simulation for the unknown parameter.

the polygenic variance set to $\sigma_u^2 = 0$. We also analyzed the mixed inheritance model (model 2) to assess the effect of the polygenic background in the estimation of Mendelian transmission probabilities. In this model, the MG additive genetic effect a, MG dominance effect d, allele A_1 frequency p, and polygenic variance σ_u^2 were fixed to values estimated with the most plausible mixed inheritance model for plasma cortisol (best-fitting mixed inheritance model). The unknown parameters were the transmission probabilities $\tau_{A_1/A_1A_1}, \tau_{A_1/A_1A_2}, \tau_{A_1/A_2A_2}$.

Inference on Major Locus Genotypes. The strategy followed was first to determine the best-fitting mixed inheritance model for plasma cortisol. We then analyzed the mixed inheritance model (model 2) under Mendelian assumptions $\left(\tau_{A_1A_1} = 1, \tau_{A_1A_2} = 0.5, \tau_{A_2A_2} = 0\right)$.

The parameters a, d, p, and polygenic variance σ_u^2 were fixed to the values estimated for the best-fitting mixed inheritance model. This allowed the estimation of the MG locus genotype of individuals for the predetermined MG model. The software iBay, version 1.44 (Janss, 2008) internally stores the genotype as the genotype number 1, 2, or 3 ($1 = A_1A_1$, $2 = A_1A_2$, $3 = A_2A_2$). During cycles of the MCMC, one of these values will be selected for every genotype and averaged over several MCMC cycles. This produces a genotype estimate that is a fractional number. The resulting estimate will be close to 1 when the genotype is most likely "1" and close to 3 when the genotype is most likely "3." Genotype estimates around 2 are somewhat ambiguous because this can correspond to a likely heterozygote, but also to a relatively inaccurate genotype that has been averaged. Thus, to safeguard against this potential drawback, we estimated the accuracy (R) for the posterior mean of a genotype (g_i) or predicted the MG locus genotype, defined as

$$R = 1 - \left\{\frac{\left[PSD\left(g_i\right)\right]^2}{\sigma_{g_0}^2}\right\},$$

where $PSD(g_i)$ is the posterior standard deviation (**PSD**) for g_i; the expected posterior variance for a genotype is

$$\sigma_{g_0}^2 = \left\{1\left(p^2\right) + 4\left[2p\left(1-p\right)\right] + 9\left(1-p\right)^2\right\} - \left[E\left(g_0\right)\right]^2;$$

and the expected genotype is

$$E\left(g_0\right) = \left\{1\left(p^2\right) + 2\left[2p\left(1-p\right)\right] + 3\left(1-p\right)^2\right\}.$$

RESULTS

Polygenic Model

Inferences for a polygenic model were obtained for the full data set using the polygenic inheritance model (model 1). Analysis of the 30 Gibbs chains with virtually 60 independent samples per chain indicated good convergence; the scale reduction factor reached $\hat{R} = 1.0$ for all the variance components and for the heritability estimate of plasma cortisol (Table 2). The posterior means of variance components and polygenic heritabil-

ity are presented in Table 2. The estimated SD of the marginal posterior distributions of these variance components indicated that these estimates were significant. The estimated polygenic variance and heritability suggest that there is a reasonable amount of genetic variance for plasma cortisol in the population under study. The polygenic heritability estimate of $h_p^2 = 0.26$ was significant and likely was different from zero, as suggested by the $HPDR_{95}$ boundaries of 0.18 and 0.35, respectively.

Mode of Inheritance of Plasma Cortisol

A summary of estimated marginal posterior means for variance components and major locus parameters using nongenetic, polygenic, and mixed inheritance models of plasma cortisol is presented in Table 3. Comparing the $\log_e [p(y|H_i)]$ of the tested models, we could not reject the hypothesis of no polygenic background [model tested: 1 vs. 2; BF $(H_2; H_1) = 0.0$]. However, there was strong evidence to support the polygenic background or polygenic heritability model when compared with the sporadic or nongenetic effects model [model tested: 4 vs. 3; BF $(H_2; H_1) = 11.8$]. These model comparisons and results of Table 2 support the contribution of polygenic background effects in plasma cortisol variation.

To infer Mendelian transmission of an MG, 3 model comparisons were sequentially performed. First, the hypothesis of no MG effects was rejected [model tested: 2 vs. 3; BF $(H_2; H_1) = 7.5$]. Second, the hypothesis of an environmental model could be ruled out because all the estimated transmission probabilities (although far from the expected Mendelian proportions) clearly did not overlap (model 1). For the general fixed model with $\sigma_u^2 = 0$, the estimated transmission probabilities were closer to the expected Mendelian proportions (model 2). This indicates that there was genetic transmission of plasma cortisol, but this trait might have a complex mode of inheritance and might be controlled by more than 1 MG. Third, all the mixed inheritance models were rejected (model tested: 5 to 8 vs. 1), which suggests a lack of statistical support for a purely Mendelian transmission of 1 MG and polygenic background in the inheritance of plasma cortisol. Although the hypothesis of mixed inheritance of 1 dominant cortisol-decreasing allele A_1 and polygenic background (model 7) was rejected [model tested: 7 vs. 1; BF $(H_2; H_1) = -1.8$], model 7 had one of the best $\log_e [p(y|H_i)]$ estimates among the mixed inheritance models (models 5 to 8). Furthermore, according to the guidelines provided by Janss (2008) for judging the difference between 2 competing models, model 1 was only marginally better than model 7.

When comparing model 7 with the second best-fitting mixed inheritance model of 1 additive MG and a polygenic background (model 6), according to the guidelines proposed by Janss (2008), there was substantial evidence in favor of the mixed inheritance model 7

Table 3. Estimated marginal posterior means for variance components and major gene parameters of plasma cortisol when using polygenic and mixed inheritance models in Bayesian segregation analysis[1]

Model[2]	σ_e^2	σ_u^2	σ_p^2	σ_g^2	a	d	p	τ_{A_1/A_1A_1}	τ_{A_1/A_1A_2}	τ_{A_1/A_2A_2}	h_p^2	$\text{Log}_e [p(y/H_i)]^3$	Model tested	$\text{BF}(H_2; H_1)^4$	Total chains
1. General	616.1	182.7	26.6	6,8436.5	129.4	−7.5	0.60	0.97	0.87	0.53	0.23	−7,077.5	1 vs. 2	0.0	30
2. General fixed	616.2	[0][5]	140.3	485.8	20.4	−7.7	0.49	0.98	0.70	0.05	[0]	−7,077.4	2 vs. 3	7.5	34
3. Sporadic	618.1	[0]	376.4	[0]	[0]	[0]	[0.0]	[0]	[0]	[0]	[0]	−7,084.9	4 vs. 3	11.8	37
4. Polygenic	617.6	223.9	131.7	[0]	[0]	[0]	[0.0]	[0]	[0]	[0]	0.26	−7,073.2	7 vs. 6	3.3	30
5. Dominant A_2	616.1	177.2	129.3	13.4	7.8	7.8	0.67	[1]	[0.5]	[0]	0.22	−7,087.7	5 vs. 1	−10.2	30
6. Additive	618.7	66.9	121.7	471.5	31.4	[0]	0.41	[1]	[0.5]	[0]	0.10	−7,082.6	6 vs. 1	−5.1	33
7. Dominant A_1	620.7	280.1	35.3	1,649.3	41.7	−41.7	0.74	[1]	[0.5]	[0]	0.31	−7,079.3	7 vs. 1	−1.8	30
8. Codominant	619.7	265.4	32.0	1,790.4	44.6	−41.5	0.74	[1]	[0.5]	[0]	0.30	−7,095.3	8 vs. 1	−17.9	32

[1]Bayesian segregation analysis of plasma cortisol (ng/mL) was performed with the software iBay, version 1.44 (Janss, 2008). The Gibbs sampler had these characteristics: number of iterations per chain = 1,200,000; burn-in period = 600,000; thinning = 10,000; collected samples per chain = 60; total chains >30; and total collected samples >1,800.

[2]Model parameters: error variance, σ_e^2; polygenic variance, σ_u^2; permanent environmental variance, σ_p^2; major gene variance, σ_g^2. Major gene additive effect, a; major gene dominance effect, d; frequency of cortisol-decreasing allele A_1, p; polygenic model heritability, h_p^2, defined as $\sigma_u^2 / \left(\sigma_u^2 + \sigma_e^2\right)$; and transmission probabilities $\tau\left(\tau_{A_1/A_1A_1}, \tau_{A_1/A_1A_2}, \tau_{A_1/A_2A_2}\right)$, defined as the probability that a parent with any of the three genotypes (A_1A_1,A_1A_2,A_2A_2) transmits the allele A_1 to its offspring.

[3]$\log_e$ of the marginal density under the fitted model H_i.

[4]Bayes factor $\text{BF}(H_2; H_1) = p(y/H_2)/p(y/H_1)$ is the ratio of the marginal likelihood under 1 model to the marginal likelihood under a second model, and H_1 and H_2 are the 2 competing models.

[5]Values between squared brackets indicate the parameter was fixed to the value shown.

[model tested: 7 vs. 6; BF (H_2; H_1) = 3.3]. Definitely, the mixed inheritance models 5 and 8 could be ruled out because they had the worst $\log_e [p(y|H_i)]$ estimates among the 4 mixed inheritance models. Overall, these results suggest that the inheritance of plasma cortisol might be controlled by 1 or more dominant cortisol-decreasing MG alleles and polygenic background effects in the sampled population.

Evidence of Major Genes Affecting Plasma Cortisol

The estimated marginal posterior mean, PSD, and left and right $HPDR_{95}$ for variance components and MG parameters of plasma cortisol for all of the evaluated models, with the exception of the polygenic and sporadic models, are presented in Table 4.

For the most plausible mixed inheritance model for plasma cortisol, dominant cortisol-decreasing allele A_1 and polygenic background effects (dominant A_1 model), the posterior distribution of the MG variance $\left(\sigma_g^2\right)$ for plasma cortisol is presented in Figure 1. The σ_g^2 is significant because the $HPDR_{95}$ for its variance does not include 0 (Table 4). Similarly, the posterior distribution of additive variance attributable to the MG $\left(\sigma_a^2\right)$ and the polygenic variance $\left(\sigma_u^2\right)$ for plasma cortisol (Figure 1) are significant because the $HPDR_{95}$ for both variances do not include 0 (Table 4). The posterior mean and PSD for the additive effect $\hat{a}$ are −41.7 ng/mL and 4.8, respectively. The additive effect $\hat{a}$ in genetic SD units was $a_{GSD} = 0.96 \pm 0.05$, which suggests that this is a relatively large MG with a significant effect. The posterior distribution of $\hat{a}$ is presented in Figure 2, and it is clearly significant because its $HPDR_{95}$ does not include 0 (Table 4). Finally, the posterior mean of the dominant cortisol-decreasing allele frequency was $p_{A_1} = 0.74$ (Table 4), which is also significant because its $HPRD_{95}$ does not include 0 (Figure 3).

For the codominant model, the results confirm the presence of a dominant cortisol-decreasing allele A_1, with dominance effect $d = -41.5$ and frequency $p_{A_1} = 0.74$. Partial dominance can be rejected, because the $HPDR_{95}$ for the difference between the additive effect and the absolute dominance effect $\left(a - |d|\right)$ covers 0, so complete dominance of the A_1 allele can be accepted (data not shown).

In Table 4, we also present results on within- and between-chain variances, the effective number of samples, and the scale reduction factor $\hat{R}$ for each estimated scalar parameter within each model evaluated. These results indicate that mixing and convergence of the MCMC iterations are very good for all the mixed inheritance models. However, for the general and general fixed models, we acknowledge that the convergence for several parameters is still problematic. Approximate convergence was reached only for 5 of 13 and 2 of 7 parameters in the general and general fixed models, respectively.

Inference on Transmission Probabilities

For further validation of evidence on MG for plasma cortisol, transmission probabilities were estimated by fitting MG and mixed inheritance models in the BSA (Table 5). For the best-fitting MG model and the best-fitting mixed inheritance model, the left-right bounds of $HPDR_{95}$ for the first and last transmission probability do not overlap. It is interesting to note that the transmission probabilities resembled Mendelian transmission more closely when fitting a general fixed model $\left(\sigma_u^2 = 0\right)$. Under this model, the posterior mean (and PSD) transmission probabilities were 0.98 (0.01), 0.70 (0.28), and 0.05 (0.05), respectively. For this general fixed model, the left-right bounds of $HPDR_{95}$ for the first and last transmission probabilities clearly do not overlap.

When the MG and polygenic effects are set to values estimated with the most plausible mixed inheritance model for plasma cortisol (model 7), the 3 transmission probabilities reached very good convergence $\left(\hat{R} = 1.0\right)$ for the best-fitting MG and best-fitting mixed inheritance models (Table 5). However, approximate convergence of the simulated iterations was reached for only 1 of 3 of the transmission probabilities when fitting the general fixed model (Table 5).

Overall, because 2 of the transmission probabilities do not overlap, an environmental hypothesis for the inferred mixture distribution for plasma cortisol can be rejected. In this case, there is proof of a genetic transmission of some kind in the inheritance of plasma cortisol, although it may not be strictly Mendelian. The distortion can be of genetic origin (i.e., the MG may be X-linked), the MG can have multiple alleles, or the true model can be digenic, and this can result in an apparent distortion in the Mendelian transmission when the data are fitted using an autosomal biallelic model. Thus, it can be accepted that a simple autosomal dominant gene is not the most plausible mode of inheritance for plasma cortisol.

Inference on Major Gene Genotypes

The BSA enabled estimation of the posterior probability of major locus genotypes for each pedigree member. Here, we present only the predicted major locus genotypes for parents of each family (Table 6) when using the most plausible mixed inheritance model for plasma cortisol (dominant cortisol-decreasing allele A_1 and polygenic effects) from the BSA (Table 3). The predicted major locus genotypes suggested that we had 4 F_1 intercross families (18, 58, 60, and 125) and 1

Table 4. Estimated marginal posterior mean, SD, and 95% highest posterior density region for variance components[1] of plasma cortisol when using mixed inheritance model in Bayesian segregation analysis[2]

Model and statistic	σ_e^2	σ_u^2	σ_g^2	σ_p^2	a	d	p	a_{GSD}	σ_a^2	R_g	R_u	R_{gu}	h_p^2
General													
Mean	616.1	182.7	68,436.5	26.6	129.4	−7.5	0.60	1.2	22,595.3	0.53	0.10	0.63	0.23
SD	22.0	54.0	247,749.9	22.7	275.8	308.3	0.22	0.3	80,267.1	0.25	0.06	0.19	0.05
Left $HPDR_{95}$[3]	573.4	88.9	0.0	0.0	0.0	−761.7	0.17	0.4	−22,298.1	0.14	−0.01	0.31	0.13
Right $HPDR_{95}$	660.2	294.8	543,984.1	71.7	846.5	1,223.4	0.93	1.8	175,199.1	1.03	0.20	1.03	0.33
Within-chain σ^2	478.0	2,665.5	ND[4]	425.7	9,318.6	8,857.9	0.01	0.07	ND	0.02	0.00	0.01	0.00
Between-chain σ^2	773.0	1,8391.0	ND	6,113.6	ND	ND	2.29	1.93	ND	2.73	0.12	1.71	0.02
Effective number of samples	1,113	261	7	125	4	3	8	67	10	11	22	11	286
$\hat{R}$[5]	1.0	1.0	ND	1.1	ND	ND	5.2	1.1	ND	4.1	2.1	3.9	1.0
General fixed													
Mean	616.2	[0][6]	485.8	140.3	20.4	−7.7	0.49	NE[7]	228.4	NE	[0]	NE	[0]
SD	23.1	[0]	464.5	34.3	9.8	29.8	0.14	NE	159.6	NE	[0]	NE	[0]
Left $HPDR_{95}$	570.4	[0]	64.3	81.5	1.3	−57.2	0.27	NE	−18.6	NE	[0]	NE	[0]
Right $HPDR_{95}$	661.6	[0]	1,470.0	211.1	32.0	46.4	0.82	NE	439.2	NE	[0]	NE	[0]
Within-chain σ^2	528.6	[0]	60,705.0	645.9	19.22	23.8	0.01	NE	8,072.5	NE	[0]	NE	[0]
Between-chain σ^2	746.6	[0]	ND	33,524.8	4,710.3	53,484.4	0.83	NE	ND	NE	[0]	NE	[0]
Effective number of samples	1,444	[0]	13	39	8	1	17	NE	15	NE	[0]	NE	[0]
$\hat{R}$	1.0	[0]	ND	1.5	5.5	49.7	2.8	NE	ND	NE	[0]	NE	[0]
Additive													
Mean	618.7	66.9	471.5	121.7	31.4	[0]	0.41	1.4	471.5	0.37	0.05	0.42	0.10
SD	23.3	28.1	97.6	26.7	3.1	[0]	0.08	0.1	97.6	0.05	0.02	0.05	0.04
Left $HPDR_{95}$	571.5	19.0	284.0	70.8	25.3	[0]	0.26	1.2	284.0	0.27	0.01	0.32	0.03
Right $HPDR_{95}$	662.9	123.8	660.0	176.4	37.3	[0]	0.56	1.5	660.0	0.46	0.09	0.51	0.17
Within-chain σ^2	541.5	790.4	9,442.2	708.7	9.52	[0]	0.01	0.00	9,442.2	0.00	0.00	0.00	0.00
Between-chain σ^2	564.2	776.0	14,570.3	902.1	16.2	[0]	0.01	0.00	14,570.3	0.00	0.00	0.00	0.00
Effective number of samples	1,900	2,017	1,283	1,556	1,165	[0]	1,482	1,583	1,283	1,310	1,710	1,620	2,101
$\hat{R}$	1.0	1.0	1.0	1.0	1.0	[0]	1.0	1.0	1.0	1.0	1.0	1.0	1.0
Dominant A_1													
Mean	620.7	280.1	1,649.3	35.3	41.7	−41.7	0.74	1.0	651.9	0.63	0.11	0.74	0.31
SD	22.8	54.6	398.2	25.0	4.8	4.8	0.08	0.0	189.1	0.06	0.03	0.05	0.04
Left $HPDR_{95}$	577.1	171.6	865.6	0.0	31.9	−50.8	0.59	0.9	290.2	0.51	0.06	0.65	0.22
Right $HPDR_{95}$	667.5	384.2	2,424.8	83.5	50.8	−31.9	0.89	1.1	1,031.1	0.74	0.16	0.82	0.39
Within-chain σ^2	521.4	2,990.9	157,550.8	621.8	22.9	22.8	0.01	0.00	35,649.8	0.00	0.00	0.00	0.00
Between-chain σ^2	484.5	2,367.7	220,201.8	680.1	26.9	26.9	0.00	0.00	43,370.3	0.01	0.00	0.00	0.00
Effective number of samples	1,937	2,274	1,288	1,646	1,531	1,531	2,293	1,687	1,480	1,281	1,939	1,223	2,214
$\hat{R}$	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
Dominant A_2													
Mean	616.1	177.2	13.4	129.3	7.8	7.8	0.67	0.6	22.8	0.01	0.19	0.20	0.22
SD	23.4	65.3	17.1	30.8	5.0	5.0	0.28	0.5	22.0	0.02	0.06	0.06	0.06
Left $HPDR_{95}$	570.5	56.1	0.0	69.5	0.0	−0.4	0.09	−0.1	−5.4	0.00	0.07	0.09	0.09

Continued

Table 4 (Continued). Estimated marginal posterior mean, SD, and 95% highest posterior density region for variance components[1] of plasma cortisol when using mixed inheritance model in Bayesian segregation analysis[2]

Model and statistic	σ_e^2	σ_u^2	σ_g^2	σ_p^2	a	d	p	a_{GSD}	σ_a^2	R_g	R_u	R_{gu}	h_p^2
Right $HPDR_{95}$	661.0	303.4	44.0	187.4	15.1	15.5	1.00	1.4	65.2	0.05	0.30	0.31	0.34
Within-chain σ^2	547.8	4,287.3	290.1	948.2	25.4	25.4	0.08	0.22	485.1	0.00	0.00	0.00	0.00
Between-chain σ^2	425.5	2,696.0	346.5	991.8	22.0	22.0	0.10	0.15	397.3	0.00	0.00	0.00	0.00
Effective number of samples	2,317	2,862	1,507	1,721	2,075	2,075	1,332	2,599	2,198	1,534	2,857	3,861	3,300
$\hat{R}$	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
Codominant													
Mean	619.7	265.4	1,790.4	32.0	44.6	−41.5	0.74	1.0	767.3	0.65	0.10	0.75	0.30
SD	22.7	54.0	484.6	25.0	8.8	5.8	0.08	0.1	326.8	0.07	0.03	0.05	0.04
Left $HPDR_{95}$	575.7	164.8	854.6	0.0	28.5	−53.2	0.59	0.8	179.5	0.51	0.05	0.65	0.21
Right $HPDR_{95}$	665.2	373.0	2,736.1	81.1	62.2	−30.0	0.90	1.2	1,410.9	0.77	0.16	0.84	0.38
Within-chain σ^2	514.6	2,928.4	233,603.2	624.3	77.0	33.2	0.01	0.01	106,040.7	0.00	0.00	0.00	0.00
Between-chain σ^2	546.1	2,042.6	312,498.7	684.7	115.3	32.4	0.01	0.02	151,554.7	0.00	0.00	0.00	0.00
Effective number of samples	1,809	2,753	1,435	1,751	1,282	1,964	2,116	1,405	1,343	1,809	1,760	2,189	2,682
$\hat{R}$	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0

[1]Parameters: error variance, σ_e^2; polygenic variance, σ_u^2; major gene variance, σ_g^2; permanent environmental variance, σ_p^2; major gene additive effect, a; major gene additive effect in genetic SD units, a_{GSD}; major gene dominance effect, d; frequency of cortisol-decreasing allele A_1, p; additive variance attributable to major gene, σ_a^2; proportion of total variance attributable to the total effect of major gene, R_g, defined as $\sigma_g^2 / \left(\sigma_g^2 + \sigma_u^2 + \sigma_p^2 + \sigma_e^2\right)$; proportion of total variance attributable to polygenic effects, R_u, defined as $\sigma_u^2 / \left(\sigma_g^2 + \sigma_u^2 + \sigma_p^2 + \sigma_e^2\right)$; proportion of total variance attributable to genetic effects, R_{gu}, defined as $\left(\sigma_g^2 + \sigma_u^2\right) / \left(\sigma_g^2 + \sigma_u^2 + \sigma_p^2 + \sigma_e^2\right)$; and polygenic model heritability, h_p^2, defined as $\sigma_u^2 / \left(\sigma_u^2 + \sigma_e^2\right)$.

[2]Bayesian segregation analysis of plasma cortisol (ng/mL) was performed with the software iBay, version 1.44 (Janss, 2008). The Gibbs sampler had these characteristics: number of iterations per chain = 1,200,000; burn-in period per chain = 600,000; thinning = 10,000; collected samples per chain = 60; total chains >30; and total collected samples >1,800.

[3]$HPDR_{95}$ is the 95% highest posterior density region.

[4]ND indicates not determined because of a large within-chain (between-chain) variance estimate, which leads to a large $\hat{R}$ estimate, suggesting poor convergence of the iterative simulation for this scalar parameter.

[5] $\hat{R}$ is the scale reduction factor. The $\hat{R}$ should be approximately 1.0 (or at most ≤1.1) to indicate convergence of the iterative simulation for the unknown parameter.

[6]Values between brackets indicate the parameter was fixed to the value shown.

[7]NE indicates not estimated because of the model constraint of null polygenic variance effect $\left(\sigma_u^2 = 0\right)$.

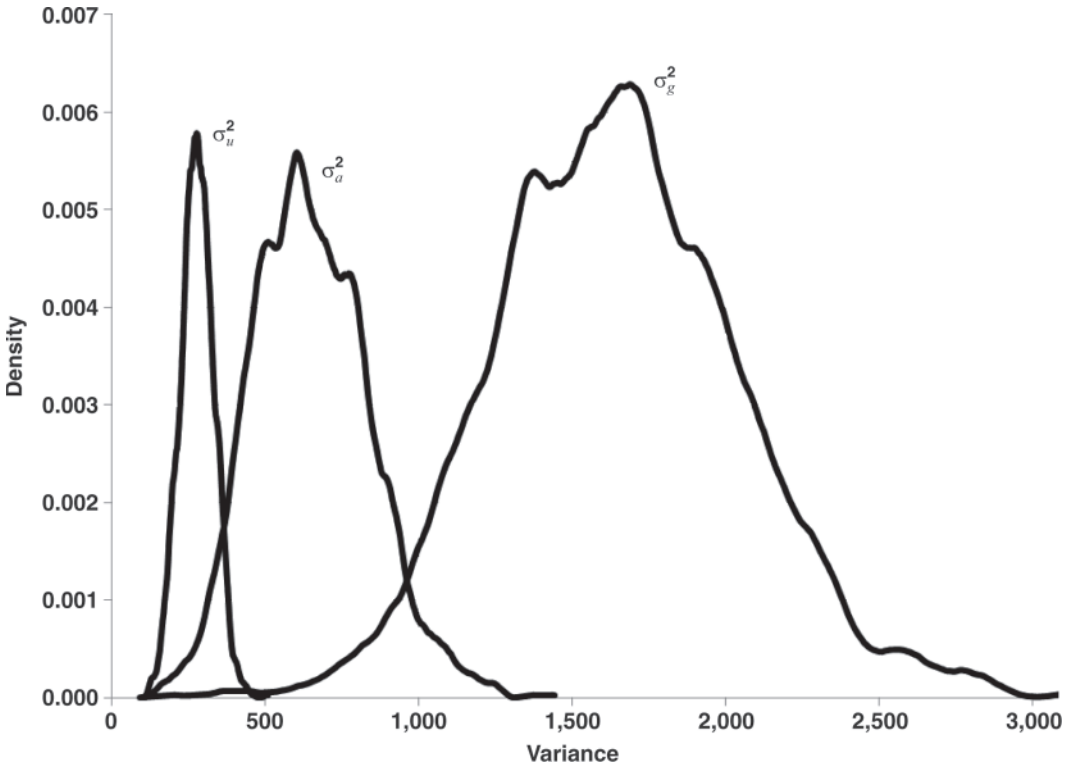

Figure 1. Estimated marginal posterior distribution of polygenic variance (σ_u^2), total major gene variance (σ_g^2), and additive genetic variance attributable to the major gene (σ_a^2) from Bayesian segregation analysis of plasma cortisol in rainbow trout broodstock. A mixed inheritance model of a major locus with a dominant cortisol-decreasing allele A_1 and polygenic effects was used in the Bayesian segregation analysis with the computer program iBay, version 1.44 (Janss, 2008). The Gibbs sampler had these characteristics: number of iterations per chain = 1,200,000; burn-in period per chain = 600,000; thinning = 10,000; collected samples per chain = 60; total chains >30; and total collected samples >1,800.

backcross family (126), although the genotype of the dam in family 126 had low accuracy. In the F_1 intercross families, siblings with high accuracy for the predicted genotype can be identified and brother-sister can be mated to generate F_2 families for mapping QTL for stress response. Based on the inferred likely mode of inheritance of plasma cortisol in the sampled population, it will be most efficient to intercross siblings from the F_1 intercross families with homozygous individuals for the cortisol-increasing allele A_2 to generate backcross families that can be used in QTL mapping for stress response in this rainbow trout population. We also noticed that there was a significant correlation of the posterior mean of a genotype with the plasma cortisol mean in parents ($r = 0.85$, $P = 0.0001$; data not shown). This indicates high correspondence between the plasma cortisol phenotypes of the parents and their predicted major locus genotypes.

DISCUSSION

In this study, we found that the Bayesian CSA is a powerful and informative method of analysis to detect MG effects on plasma cortisol variation in rainbow trout. The CSA provided information on the mode of inheritance of plasma cortisol, the magnitude of MG and polygenic background effects, and the heritability of plasma cortisol in the studied population.

The results from this study clearly support a familial transmission and evidence of segregating MG for stress response, a potentially economically important trait in rainbow trout. The results from BSA for plasma cortisol provided significant statistical evidence supporting the view that at least 1 MG with a dominant cortisol-decreasing allele and polygenic background effects is likely underlying the genetic variation of stress response in the sampled population.

The estimated magnitude of the MG effect in genetic SD units was $a_{GSD} = 0.96 \pm 0.05$ when assuming a mixed inheritance model of an MG dominant cortisol-decreasing allele and polygenic background effects in BSA (dominant A_1 model). These results suggest that there must be genes of relatively large effect for stress response segregating in the sampled population, which might explain approximately 63% of the total stress response variance.

It is clear from these results that the stress response in rainbow trout is hereditary and is influenced by genetic factors transmitted from parents to offspring. The results obtained here indicate that stress response is likely determined by the interplay of one or more dominant cortisol-decreasing MG, a large number of minute-effect additive polygenes, environmental factors, and their interactions. This study also supports the importance of polygenic effects in the genetic variation of stress response in the studied population. The magni-

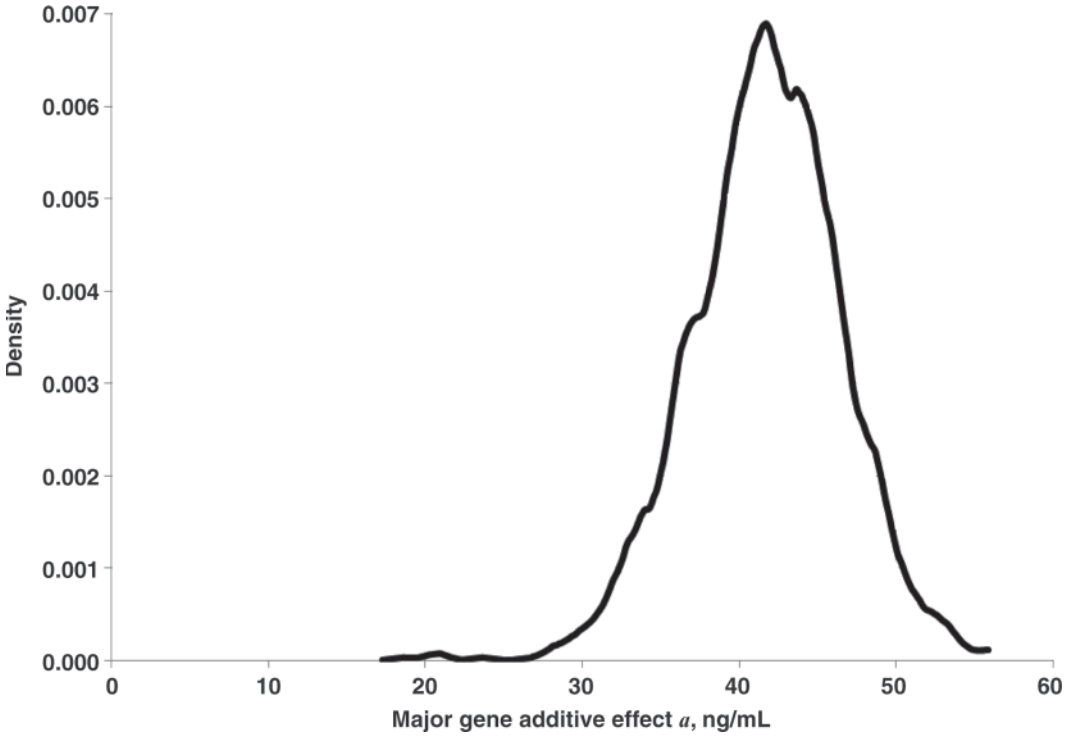

Figure 2. Estimated marginal posterior distribution of the major gene additive effect (a) from Bayesian analysis of plasma cortisol in rainbow trout broodstock. A mixed inheritance model of a major locus with a dominant cortisol-decreasing allele A_1 and polygenic effects was used in the Bayesian segregation analysis with the computer program iBay, version 1.44 (Janss, 2008). The Gibbs sampler had these characteristics: number of iterations per chain = 1,200,000; burn-in period per chain = 600,000; thinning = 10,000; collected samples per chain = 60; total chains >30; and total collected samples >1,800.

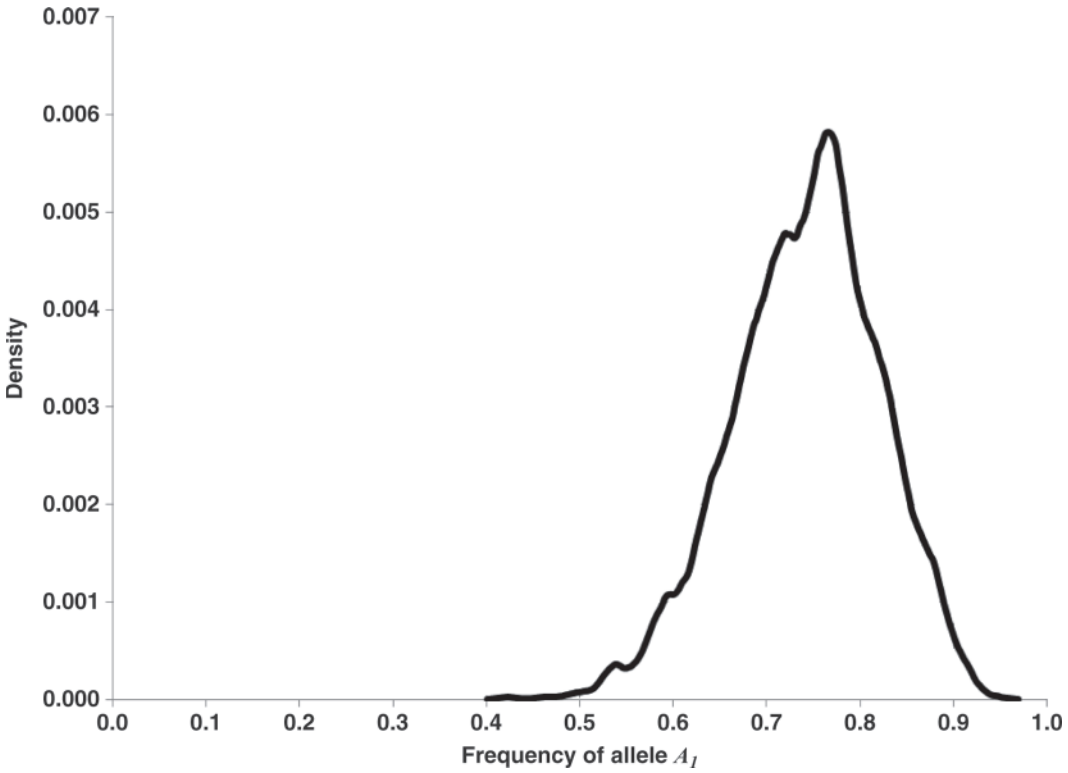

Figure 3. Estimated marginal posterior distribution of major locus cortisol-decreasing allele (A_1) from Bayesian segregation analysis of plasma cortisol in rainbow trout broodstock. A mixed inheritance model of a major locus with a dominant cortisol-decreasing allele A_1 and polygenic effects was used in the Bayesian segregation analysis with the computer program iBay, version 1.44 (Janss, 2008). The Gibbs sampler had these characteristics: number of iterations per chain = 1,200,000; burn-in period per chain = 600,000; thinning = 10,000; collected samples per chain = 60; total chains >30; and total collected samples >1,800.

Table 5. Mendelian transmission probabilities estimated from the marginal posterior distributions after Bayesian segregation analysis[1] of plasma cortisol in rainbow trout broodstock

	General fixed model[2]			Best-fitting MG model[3]			Best-fitting mixed inheritance model[4]		
Statistic	τ_{A_1/A_1A_1}	τ_{A_1/A_1A_2}	τ_{A_1/A_2A_2}	τ_{A_1/A_1A_1}	τ_{A_1/A_1A_2}	τ_{A_1/A_2A_2}	τ_{A_1/A_1A_1}	τ_{A_1/A_1A_2}	τ_{A_1/A_2A_2}
Mean	0.98	0.70	0.05	0.97	0.83	0.53	0.98	0.89	0.57
SD	0.01	0.28	0.05	0.03	0.11	0.23	0.02	0.11	0.24
Left $HPDR_{95}$[5]	0.96	0.08	0.00	0.92	0.62	0.07	0.94	0.71	0.07
Right $HPDR_{95}$	1.00	1.00	0.14	1.00	0.99	0.90	1.00	1.00	0.93
Within-chain σ^2	0.0002	0.0102	0.0008	0.0006	0.0113	0.0532	0.0004	0.0113	0.0590
Between-chain σ^2	0.0020	4.0841	0.0826	0.0006	0.0116	0.0534	0.0004	0.0181	0.0680
Effective number of samples	146	5	20	1,321	1,167	1,194	1,091	752	1,040
$\hat{R}$[6]	1.0	8.9	2.5	1.0	1.0	1.0	1.0	1.0	1.0

[1]Bayesian segregation analysis of plasma cortisol (ng/mL) was performed with the software iBay, version 1.44 (Janss, 2008). The Gibbs sampler had these characteristics: number of iterations per chain = 1,200,000; burn-in period per chain = 600,000; thinning = 10,000; collected samples per chain = 60; total chains = 20; and total collected samples = 1,200.

[2]General fixed model. The polygenic variance was fixed to $\sigma_u^2 = 0$. The unknown parameters were as follows: major gene additive effect, a; major gene dominance effect, d; frequency of cortisol-decreasing allele A_1, p; major gene variance, σ_g^2; error variance, σ_e^2; permanent environmental variance, σ_p^2; and transmission probabilities, $\tau_{A_1/A_1A_1}, \tau_{A_1/A_1A_2}, \tau_{A_1/A_2A_2}$.

[3]Best-fitting major genes (MG) model. The MG parameters a, d, and p were fixed to values estimated with the best-fitting mixed inheritance model (dominant cortisol-decreasing allele A_1 and polygenic effects), and polygenic variance was fixed to $\sigma_u^2 = 0$. The unknown parameters were $\tau_{A_1/A_1A_1}, \tau_{A_1/A_1A_2}, \tau_{A_1/A_2A_2}$.

[4]Best-fitting mixed inheritance model. The MG parameters a, d, p, and σ_u^2 were fixed to values estimated with the best-fitting mixed inheritance model. The unknown parameters were $\tau_{A_1/A_1A_1}, \tau_{A_1/A_1A_2}, \tau_{A_1/A_2A_2}$.

[5]$HPDR_{95}$ is the 95% highest posterior density region.

[6] $\hat{R}$ is the scale reduction factor. The $\hat{R}$ should be approximately 1.0 (or at most $\leq$1.1) to indicate convergence of the iterative simulation for the unknown parameter.

Table 6. Predicted major gene genotypes for parents of rainbow trout families using Bayesian segregation analysis[1]

Family	Parent identification	Sex[2]	Cortisol, ng/mL	g_i[3]	$PSD(g_i)$[4]	R[5]	Genotype	Cross type[6]
18	134552255	M	153	3.0	0.00	1.00	A_2A_2	F_1 intercross
	134473327	F	27	1.0	0.00	1.00	A_1A_1	
19	134528597	M	106	1.2	0.40	0.61	A_1A_1	
	134534543	F	34	1.4	0.47	0.45	A_1A_1	
20	133934214	M	29	1.3	0.45	0.49	A_1A_1	
	134452311	F	82	1.3	0.46	0.47	A_1A_1	
58	134728092	M	28	1.0	0.00	1.00	A_1A_1	F_1 intercross
	134633225	F	144	3.0	0.10	0.98	A_2A_2	
59	134528597	M	106	1.2	0.40	0.61	A_1A_1	
	134567133	F	36	1.3	0.47	0.46	A_1A_1	
60	133716524	M	106	3.0	0.09	0.98	A_2A_2	F_1 intercross
	133979352	F	32	1.0	0.03	1.00	A_1A_1	
62	134668746	M	109	1.3	0.44	0.51	A_1A_1	
	134626172	F	40	1.3	0.47	0.46	A_1A_1	
63	134914663	M	91	1.3	0.46	0.48	A_1A_1	
	134561470	F	38	1.3	0.46	0.48	A_1A_1	
64	134456563	M	27	1.3	0.46	0.48	A_1A_1	
	134912757	F	88	1.3	0.49	0.40	A_1A_1	
124	134927390	M	10	1.4	0.47	0.44	A_1A_1	
	133565797	F	85	1.3	0.45	0.51	A_1A_1	
125	134552255	M	153	3.0	0.00	1.00	A_2A_2	F_1 intercross
	134632746	F	26	1.0	0.00	1.00	A_1A_1	
126	134728092	M	28	1.0	0.00	1.00	A_1A_1	Backcross
	134919497	F	98	1.7	0.75	−0.41	A_1A_2	

[1]The best-fitting mixed inheritance model (dominant cortisol-increasing A_1 allele and polygenic effects) from Bayesian segregation analysis was used to predict major gene genotypes with the software iBay, version 1.44 (Janss, 2008). The Gibbs sampler had these characteristics: number of iterations per chain = 1,200,000; burn-in period per chain = 600,000; thinning = 10,000; collected samples per chain = 60; total chains = 20; and total collected samples = 1,200.

[2]M and F indicate male and female parent, respectively.

[3]Posterior mean of a genotype for the ith animal, where 1 = A_1A_1, 2 = A_1A_2, and 3 = A_2A_2.

[4]Posterior SD of a genotype for the ith animal.

[5]Accuracy for the posterior mean of a genotype (g_i).

[6]Cross type was defined using the predicted genotypes of parents within each family.

tude of the polygenic background effect had an average of $h_p^2 = 0.26$, which is slightly smaller than those previously reported in rainbow trout (Weber et al., 2008).

The implications of poor convergence observed for several parameters estimated with general and general fixed models are minimal for the conclusions drawn in this study. Poor mixing of the MCMC is a potential problem for the fit of MG models; mixing refers to the possibility of the Markov chain moving through the parameter space. For the genotypes, this implies that the chain should be able to make transitions between different genotypic configurations (Janss, 2008). Because multiple chains were run and compared and because the general model was still marginally better than the best-fitting mixed inheritance model 7, lock-up of Gibbs chains in subspaces of the parameter space likely did not occur. Further, the evidence to support significant MG and polygenic effects and variance components was based on analysis of marginal posterior distributions of polygenic and mixed inheritance models that had very good mixing and convergence in the MCMC computations.

The results presented here support a pedigree-based linkage analysis approach for the discovery of the underlying MG or QTL for crowding stress response in rainbow trout and other farm animals that might have similar models of mixed inheritance. There have been few reports of evidence for MG and QTL for stress response as measured by cortisol responsiveness in other animal species. Buitenhuis et al. (2003) detected suggestive QTL for stress response on GGA5 and GGA18 in laying hens. Desautes et al. (2002) reported highly significant gene effects for poststress cortisol concentration and a significant effect for basal cortisol concentration at the end of the q arm of chromosome 7 in pigs. Ousova et al. (2004) proposed that the corticosteroid-binding globulin gene (*Cbg*) might be the causal gene of a QTL associated with cortisol concentrations, fat deposition, and muscle content in a pig intercross. Solberg et al. (2006) identified 3 significant and 2 suggestive QTL for plasma cortisol response to a 10-min restraint stress when using divergent inbred rat strains for several hypothalamic-pituitary-adrenal axis measures. Kadarmideen and Janss (2007) detected an MG with an additive effect of 86 ng/mL of cortisol in pigs divergently selected for stress response.

Gibbs sampling allows the use of looped pedigrees, the incorporation of many relationships, and the ability to circumvent the intrinsic problems in marginalizing

a joint distribution with respect to both discrete and continuous parameters (Hasstedt, 1982). Because of these advantages, Gibbs sampling has also been used in maximum likelihood approaches to segregation analysis (Guo and Thompson, 1992). The Bayesian approach in combination with Gibbs sampling, as used in this study, provides more flexibility and accuracy than a maximum likelihood approach. For instance, in the estimation of means, they are straightforwardly included in the Gibbs chains and treated as nuisance parameters, yielding a REML-type approach by accounting for uncertainty originating from the estimation of these fixed effects (Janss et al., 1997); accounting for such uncertainty removes bias in the estimation of variance components. However, we are aware of the risk of predicting incorrect genetic models when using methods of segregation analysis that are usually not robust to violations of assumptions (Lynch and Walsh, 1997). Ultimately, the molecular approach will allow confirmation or rejection of the presence of a Mendelian locus (Jarvik, 1998).

Although the presence of a clear mode of inheritance will facilitate the genetic dissection of stress response in the rainbow trout, response in terms of plasma concentrations of cortisol is likely affected by several physiological factors, which complicate identification of genes contributing to the phenotype. As an example, our previous studies suggest that poststressor plasma cortisol was not affected by BW or length per se, but was due instead to the association between growth performance and cortisol responsiveness (Weber and Silverstein, 2007). Support for this interpretation comes from the observation that, although BW more than tripled over the 3-mo sampling period in the current study, mean plasma cortisol concentrations did not increase (mo 1 = 65.4, 2 = 50.3, 3 = 53.6, 4 = 59.4 ng/mL). The interpretation that BW per se does not affect poststressor cortisol concentrations was also drawn in studies of European lines of rainbow trout (Pottinger and Carrick, 1999a). Although treating BW and length as covariates may have caused us to misidentify genes that contribute to both stress responsiveness and growth performance in the current study, we chose to use a more simplified model to focus on identifying genes affecting stress response independent of growth characteristics. Once these stress response loci have been identified, we can attempt the complex task of identifying genes associated with the interaction of stress responsiveness and growth performance.

When mixed inheritance of an MG and polygenic background is supported, the investigator may wish to estimate the major locus genotype of particular pedigree members (i.e., QTL genotype of the parents). This is a common problem with studies of complex diseases in humans and livestock species, where pedigrees are gathered on the basis of extreme disease phenotypes. Under a mixed inheritance model, extreme individuals in some families will result from having extreme major locus genotypes, whereas in other families they will result from having extreme polygenic values (Lynch and Walsh, 1997). The information on predicted major-locus genotypes for pedigree members can be used in the design of efficient QTL mapping studies. This information on mode of inheritance of the studied trait and predicted major-locus genotypes can enable the identification of genetic mapping families (e.g., F_2 intercross and BC families), and the identification of F_1 intercross families from which the siblings can be sister-brother mated to generate large F_2 intercross or backcross mapping families. Thus, when attempting to dissect complex traits in outbred populations, we advocate early use of CSA to avoid the pain of realizing later that only a fraction of the families already genotyped on hundreds of markers are indeed informative for QTL mapping.

We are currently generating large F_2 intercross families by mating siblings from the F_1 intercross families identified in this study. These F_2 families will be used in genome-wide linkage scans for QTL affecting stress response in the NCCCWA rainbow trout broodstock. Assembly of these mapping resources provides a tool for eventually elucidating the genetic basis of variation in stress response and identifying QTL to provide more specific markers of possibly distinct stress response genotypes for phenotype association studies, for physiological studies of stress responsiveness, and possibly for selective breeding.

This study is the first of its kind in that no research so far has investigated stress response in rainbow trout by using a crowding stress paradigm to detect evidence of an MG for stress response by using Bayesian methods of CSA. The results from performing CSA support the view that one or more MG dominant cortisol-decreasing alleles and a large number of additive polygenes are likely underlying the genetic variation of plasma cortisol in the sampled rainbow trout families. These findings support a pedigree-based linkage analysis approach (and genome-wide association scans if tools and reagents are available) to the discovery of the underlying MG or QTL for stress response variation in rainbow trout.

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